AN EVALUATION OF THE STATOLITH THEORY OF GEORECEPTION IN PLANTS

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ADELARD WALTER THUENTE

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AN EVALUATION OF THE STATOLITH THEORY OF GORECEPTION IN PLANTS

Introduction

The Statolith Theory, founded in 1900 by B. Nêmec and G. Haberlandt, attempts to explain the reception of gravitational stimuli in the organs of green plants. Mobile starch grains, especially within the cells of the "columella" of the root cap and of the endodermis of stems, are regarded as "statoliths" which stimulate the sensitive plasma membrane of the cells.

As a theory, it has gained considerable recognition in Germany, less in England and other European countries, and practically none in America. No inclusive review of the subject has ever appeared in the English language.

Experimental evidence, both for and against the theory, has not been wanting. However, some of the key experiments have been questioned or reproduced with contradictory results. It was, therefore, considered worthy of effort to review, briefly, the literature on the Statolith Theory, and to repeat some of the key experiments that have been thrown into doubt. Where plausible these experiments were extended and modified in an effort to evaluate them, explain their apparent contradictions, and, on these bases, to state a modern conclusion on the validity of the theory. Other experiments were planned to study further the relationship between statolith fall and geotropic response.

Experimental Methods and Materials

All the experimental data presented were obtained from very young plants. Only coleoptiles, hypocotyls, epicotyls, and primary roots were used for study. The coleoptiles, hypocotyls, and epicotyls were obtained from seeds germinated and grown in moist sawdust. Rectangular glass dishes, four inches long, three inches deep, and one inch wide were found

most suitable for culture purposes. They were easily attached to clinostats, and permitted experimentation with several plants simultaneously. The practice followed was to plant about six to eight seeds in each dish. Since uniformity of size and vertically straight organs were desired, those not suited were pinched off, so that only three or four plants per dish were used for experimentation. The average length of *Vicia* hypocotyls used was about six centimeters, that of *Pisum* epicotyls, *Helianthus* hypocotyls, *Raphanus* hypocotyls, and *Zea* and *Avena* coleoptiles was about three to four centimeters.

The roots used in the clinostat experiments were from seeds soaked in tap water for twenty-four hours, then spread out in moist chambers on soaked filter paper. After the roots had attained a length of about two to three centimeters, they were carefully affixed to a cork disk covered with filter paper. The disk was then wedged into a small petri dish to await experimentation upon the clinostats. *Raphanus*, *Pisum* and *Zea* roots were very satisfactorily handled in this manner. A period of two hours or more was always allowed to intervene between the time of affixing the roots and the time of beginning the experiment, thus allowing the roots to reach a state of geotropic equilibrium.

Roots for the water experiments were obtained from seeds soaked in water for twenty-four hours, then placed upon a wire mesh just above the surface of the water. An electric aerator was used to bubble air through the water.

In order to obviate phototropic interference all cultural plants were kept in complete darkness. A six-watt, phototropically-inactive, red light was used when necessary for assembling the apparatus, beginning the experiments, and making the observations. The temperature at which the experimentation took place was 22°C.

Special care was given to methods for noting small curvatures. A type of "shadowgraph" was devised for observing growth and curvatures of coleoptiles, epicotyls and hypocotyls. Shadows cast by the plant organs upon graph paper, ruled in millimeters, made possible the recording of curvatures as

small as one millimeter.

To note responses in roots a celluloid measuring slide, cross-ruled in millimeters, was inserted between the root tips and the filter paper of the petri dishes.

All microscopic observations were made with hand sections of the fresh material. Iodine-potassium-iodide in aqueous solution was used to stain the starch grains.

The experimental apparatus consisted principally of several clinostats, driven by electric motors, and a "percussion machine." The slow clinostat had a variable speed from about one revolution in two minutes to one revolution in twenty minutes. The rapid clinostat was the same machine as the slow clinostat with a special "speed-up" attachment. Its speed could be varied from one revolution in six seconds to one revolution per minute. A third clinostat (speed of about one revolution in four minutes) was used to counteract the further effects of gravity after ordinary geotropic stimulation.

The "percussion machine" consisted essentially of a straight, steel bar of about three-foot length, acting as a lever. It was attached to the table at one end (fulcrum) in such a manner as to allow full freedom of upward or downward motion at the other end. A strong rubber band was attached from above, thus acting as the "resistance." The "force" was supplied by the movement of a clinostat, which, by its rotation, gave successively a downward push and a sudden, upward release action to the lever. A stop attached to the table held the bar in sudden check after the release. In this way the free end of the iron bar or lever received a series of gentle downward pushes alternated with rapid releases upward, which were terminated suddenly by the check. Experimental plants were affixed in a small bottle which was fastened near the free end of the bar. This same apparatus was arranged so that the lever was in a vertical position. In this way it was possible to subject to percussion plant organs in the vertical position also.

Experiments and Results

Series I—*Repetition of Buder's Experiments*

Buder obtained response curvatures by means of a technique through which plant organs received equilateral gravitational stimulation but unilateral statolith stimulation. His work was repeated with *Pisum sativum L.* epicotyls and roots, *Raphanus sativus L.* roots, *Zea mays L.* coleoptiles and roots, and *Avena sativa L.* coleoptiles.

The procedure was to "prepare" the plant organs by subjecting them to equal and opposite gravity stimulations so as to obtain a lateral displacement of the starch grains, without, at the same time, causing response curvatures. The plants were then placed on a rapid clinostat (one revolution in ten seconds) in such a way that the lateral cell walls upon which the starch grains had fallen during the period of the second stimulation were in a plane vertical to the face of of the clinostat. Thus during each rotation the starch grains would alternately be forced upon and released from the plasma membrane of the side upon which they had been gathered.

Of a total of 364 plant organs so treated, 56% curved in response to the lateral position of the starch grains, while 13% responded in the opposite direction (31% did not respond at all). These results agree with those of Buder, and seem to indicate a causal relationship between the fall of starch grains and georeception.

Series II — Check on *The Results of Series I*

As a check on the results of *Series I* the same species of plants were subjected to the same treatment except that the plant organs were attached to the rapid clinostat so that the lateral cell walls upon which the starch grains had gathered were in a plane parallel with the face of the clinostat. Hence the motion of the clinostat would have no further effect upon the position of the starch grains.

Of a total of 180 experimental plant organs, 14% responded with curvatures in accordance with the position of the starch grains after the "preparation" stimulation, while

12% curved in the opposite direction. These results indicate that the curvatures of *Series I* can safely be attributed to the continued action of the statoliths upon the lateral walls of the cells.

Series III—*Repetition of Zimmermann's Slow Clinostat Work*

Zimmermann showed that plant organs placed vertically to the horizontal axis of a slow clinostat (one revolution in six to twenty minutes) curved as if responding to that horizontal position which was followed by inversion. Thus during each revolution of the clinostat each plant organ is made to assume two horizontal positions. One of these is followed by a return to the normal position, the other by inversion. The responses, Zimmermann noted, were always such as would occur if the organ had been stimulated excessively in that horizontal position followed by inversion.

Pisum sativum L. epicotyls and roots, *Zea mays L.* coleoptiles and roots, *Helianthus annuus L.* hypocotyls and *Raphanus sativus L.* hypocotyls were used in repeating this work. The results were in agreement with Zimmermann's. This work is significant only in its bearing upon the following Series of experiments.

Series IV—*Repetition of Zimmermann's Geotonic Experiments*

Zimmermann concluded that inversion following horizontal stimulation increased the geotropic curvature, while the return to the normal position decreased it. Experimentation with *Lepidium sativum* roots showed that a brief period of inversion after horizontal stimulation decreased the presentation time by as much as 50%, while a brief period of normal position after horizontal stimulation increased the presentation time by as much as 50%.

The author repeated these experiments with *Pisum sativum L.* epicotyls, *Vicia faba L.* hypocotyls, *Raphanus sativus L.* hypocotyls, *Zea mays L.* and *Avena sativa L.* coleoptiles and obtained similar results. A five minute period of

inversion after horizontal stimulation decreased the presentation time from 40% to 80%, while a five minute period of normal position after horizontal stimulation increased the presentation time from 40% to 80%.

Zimmermann's interpretation of his results, in this type of experiment, as well as that of the slow clinostat, described above, was this: inversion, following horizontal displacement, would cause the statoliths to continue falling down the lateral walls of the cells, thus prolonging the stimulation. The return to normal position, on the other hand, would abruptly end statolith activity. The prolonged statolith activity caused the decrease in presentation time, while the abrupt termination of statolith activity caused an increase of presentation time.

Series V — *Repetition of the Ubisch Inversion Experiments*

Miss Ubisch repeated the work of Zimmermann with an important variation. All plant organs were inverted for a period of twenty minutes before horizontal stimulation, so that the statoliths were displaced to the morphological upper side of the cells. Hence inversion following horizontal stimulation would abruptly end statolith activity — just the opposite of the preceding experiments. Her results showed that inversion following horizontal stimulation decreased the presentation time in spite of abrupt termination of statolith activity, while the return to the normal position, in spite of prolonged statolith activity, increased the presentation time.

These experiments were repeated with *Pisum sativum L.* epicotyls, *Vicia faba L.* hypocotyls, *Raphanus sativus L.* hypocotyls, *Zea mays L.* coleoptiles and *Avena sativa L.* coleoptiles. The results were closely in agreement with those described by Miss Ubisch. Hence Zimmermann's interpretation, calling upon statolith activity to explain the fact that inversion affects geotropic response, is proved false. The results of Zimmermann cannot be regarded as proof for the Statolith Theory.

Series VI — *Destarching After Friesen and Syre* .

Friesen had obtained partial destarching in *Zea mays L.* roots and coleoptiles through the use of sulfuric acid fumes upon germinating seeds. He found a close correlation between the amounts of starch grains present and geotropic sensitivity. Syre, using similar methods, claimed complete destarching of *Zea* roots without corresponding loss of geotropic sensitivity.

The work of both these men was repeated on *Zea mays L.* roots with these results. The sulfuric acid caused destarching to varying degrees. Complete destarching occurred in no instance. Even though no so-called "statolith starch" of the root cap remained, there was always some starch in the parenchyma cells of the cortex. A close correlation between the amount of starch in the root cap and geotropic sensitivity was apparent. Furthermore, when starch grains (not visibly mobile) were present only in the parenchyma cells of the cortex, geotropic sensitivity was comparatively low.

The discrepancy between these results and those of Syre might lie in the fact that Syre seems to have disregarded the starch grains of the cortical cells.

Series VII — *Percussion Machine Experiments*

Plant organs (*Zea mays L.* coleoptiles and roots, *Raphanus sativus L.* hypocotyls, *Pisum sativum L.* epicotyls) were subjected to a series of rapid percussions so as to force the heavy, solid, cell contents to move contrary to the force of gravity.

Forty-eight percent of the plants so treated responded to the displacement of the statoliths, contrary to the force of gravity, twenty-one percent responded in accordance with gravitational force, while thirty-one percent showed no response curvatures whatever. These results are well in agreement with the tenets of the Statolith Theory.

Series VIII—*Geotropism of Zea Roots in Water Cultures*

Group 1

Normal *Zea mays L.* roots (prepared as in the preceding experiments) were grown in air and placed in a horizontal position under well-aerated water. Response, as measured by reaction time, was greatly retarded (100% — 400%). The fall of starch grains was normal. These results, as also those of the following experiments, show that geotropic response rather than sensitivity is the factor involved.

Group 2

Zea mays L. roots grown in water were placed in a horizontal position in moist chambers. The reaction time noted varied from 115 minutes to 12 hours. Examinations for statolith content showed a variety of conditions. The starch content of the root cap varied from a nearly normal amount of starch to none at all. A close correlation was found between the amount of starch grains present in the root cap and reaction time. All roots contained starch grains in the parenchyma cells of the cortex. Roots that had starch grains in the cortex only showed the longest reaction times.

Evidence was obtained to show that, upon removal from water to air, *Zea* roots increased their starch content after a period of about twelve hours. Correspondingly, geotropic sensitivity became normal after the same period of time.

Group 3

Zea mays L. roots grown in air and stimulated in air for fifteen minutes were placed in water, still in the horizontal position. The results were in all respects very similar to those from air-grown roots stimulated only in water.

Group 4

Water-grown *Zea* roots were placed horizontally in water. A correlation between starch content and decreased reaction time was again apparent.

It is evident from the results of the above mentioned experiments that geotropic response is considerably retarded when the response takes place under water. However, in spite

of the retarded response, a close correlation exists between starch content and reaction time.

Discussion

The experiments here recorded agree in support of the Statolith Theory. A very close correlation exists between the fall of starch grains (as demonstrated by earlier workers and by the author) and geotropic response. No satisfactory refutation of the Statolith Theory has ever been produced. Hence it is concluded that starch grains are causally connected with geotropic sensitivity.

Recent advances in regard to "growth-substances" and their relation to response curvatures, in no way conflict with the Statolith Theory. That growth-substances are unilaterally redistributed by gravitational stimuli has been well established. However, the mechanism through which this takes place is unknown. The Statolith Theory postulates that the first internal reception of the gravitational stimulus is due to the fall and consequent pressure of starch grains. Perhaps some causal relationship exists between statolith pressure and auxin redistribution.

VITA

Adelard Walter Thuente was born June 6, 1910, at Festina, Iowa.

He received his elementary training in the parochial schools of Festina, Iowa, and Hoven, South Dakota, and his secondary education at St. John's High School at Collegeville, Minnesota.

During the years 1930-1934 he attended St. John's University at Collegeville, Minnesota, and received the Bachelor of Arts degree with a major in philosophy. In the meantime (1932) he had been received into the Order of St. Benedict, at Collegeville, Minnesota.

He completed the theological course at St. John's Seminary in 1938 and was ordained to the Catholic priesthood in the spring of that year.

During the summer of 1939 he entered the Graduate School of Minnesota University, and transferred to the University of Illinois in the fall of 1939. The Master of Science degree was received there in the summer of 1940.

The years 1940-1942, including the summer session of 1941, were spent at the University of Illinois.

He is the junior author of "Some Quantitative Aspects of Phototropism," *Illinois Academy of Science Transactions,* 34 (2) : 86-89 (By Fuller, H. J. and————————).